Nocturnal gamma-hydroxybutyrate reduces cortisol awakening response and morning kyrunenine pathway metabolites in healthy volunteers

Dornbierer, Dario A ; Boxler, M ; Voegel, C D ; Stucky, Benjamin ; Steuer, A E ; Binz, T M ; Baumgartner, M R ; Baur, Diego M ; Quednow, B B ; Kraemer, T ; Seifritz, E ; Landolt, Hans-Peter ; Bosch, O G

Abstract: Background Gamma-hydroxybutyrate (GHB; or sodium oxybate) is an endogenous GHB-/GABAB receptor agonist. It is approved for the application in narcolepsy and has been proposed for potential treatment of Alzheimer's and Parkinson's disease, fibromyalgia, and depression, all of which involve neuro-immunological processes. Tryptophan catabolites (TRYCATs), the cortisol awakening response (CAR), and brain derived neurotrophic factor (BDNF) have been suggested as peripheral biomarkers of neuropsychiatric disorders. GHB has been shown to induce a delayed reduction of T helper and natural killer cell counts and alter basal cortisol levels, but GHB's effects on TRYCATs, CAR and BDNF are unknown. Methods Therefore, TRYCAT and BDNF serum levels as well as CAR and the affective state (Positive and Negative Affect Schedule, PANAS) were measured in the morning after a single nocturnal dose of GHB (50 mg/kg body weight) in 20 healthy male volunteers in a placebo-controlled, balanced, randomized, double-blind, cross-over design. Results In the morning after nocturnal GHB administration, the TRYCATs indolelactic acid, kynurenine, kynurenic acid, 3-hydroxykynurenine, and quinolinic acid, the 3-hydroxykynurenine to kynurenic acid ratio and the CAR were significantly reduced ($p<0.05$-0.001, Benjamini-Hochberg corrected). The quinolinic acid to kynurenic acid ratio was reduced by trend. Serotonin, tryptophan, and BDNF levels as well as PANAS scores in the morning remained unchanged after nocturnal GHB challenge. Conclusions GHB has postacute effects on peripheral biomarkers of neuropsychiatric disorders, which might be a model to explain some of its therapeutic effects in disorders involving neuro-immunological pathologies (ClinicalTrials.gov: NCT02342366).

DOI: https://doi.org/10.1093/ijnp/pyz047

Nocturnal gamma-hydroxybutyrate reduces cortisol awakening response and morning kynurenine pathway metabolites in healthy volunteers

Dornbierer DA[1,2,3,5,*], MSc; Boxler M[3], PhD; Voegel CD[4], MSc, Stucky B[1,5], PhD; Steuer AE[3], PhD; Binz TM[4], PhD; Baumgartner MR[4], PhD; Baur DM[1,5], MSc; Quednow BB[2,6], PhD; Kraemer T[3], PhD; Seifritz E[2,5,6], MD; Landolt HP[1,5,6,**], PhD; Bosch OG[2,**], MD

[1] *Institute of Pharmacology and Toxicology, University of Zurich*

[2] *Department of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric Hospital, University of Zurich*

[3] *Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich*

[4] *Center for Forensic Hair Analytics, Zurich Institute of Forensic Medicine, University of Zurich*

[5] *Sleep & Health Zurich, University Center of Competence, University of Zurich*

[6] *Neuroscience Center Zurich, University Zurich and Swiss Federal Institute of Technology (ETH) Zurich*

** *both authors contributed equally to this work*

Conflict of interest: all authors declare no conflict of interest

Original research article to be submitted to

International Journal of Neuropsychopharmacology

Version

March 18, 2019

***Corresponding author:**

Dario A. Dornbierer, MSc

Experimental and Clinical Pharmacopsychology
Department of Psychiatry, Psychotherapy and Psychosomatics
Psychiatric Hospital, University of Zurich
Lenggstrasse 31, CH-8032 Zurich, Switzerland

Phone: +41-44-384-2777
Fax : +41-44-383-3396

E-Mail: dornbierer@pharma.uzh.ch

Abstract

Background. Gamma-hydroxybutyrate (GHB; or sodium oxybate) is an endogenous GHB-/$GABA_B$ receptor agonist. It is approved for the application in narcolepsy and has been proposed for potential treatment of Alzheimer's and Parkinson's disease, fibromyalgia, and depression, all of which involve neuro-immunological processes. Tryptophan catabolites (TRYCATs), the cortisol awakening response (CAR), and brain derived neurotrophic factor (BDNF) have been suggested as peripheral biomarkers of neuropsychiatric disorders. GHB has been shown to induce a delayed reduction of T helper and natural killer cell counts and alter basal cortisol levels, but GHB's effects on TRYCATs, CAR and BDNF are unknown.

Methods. Therefore, TRYCAT and BDNF serum levels as well as CAR and the affective state (Positive and Negative Affect Schedule, PANAS) were measured in the morning after a single nocturnal dose of GHB (50 mg/kg body weight) in 20 healthy male volunteers in a placebo-controlled, balanced, randomized, double-blind, cross-over design.

Results. In the morning after nocturnal GHB administration, the TRYCATs indolelactic acid, kynurenine, kynurenic acid, 3-hydroxykynurenine, and quinolinic acid, the 3-hydroxykynurenine to kynurenic acid ratio and the CAR were significantly reduced ($p<0.05$-0.001, Benjamini-Hochberg corrected). The quinolinic acid to kynurenic acid ratio was reduced by trend. Serotonin, tryptophan, and BDNF levels as well as PANAS scores in the morning remained unchanged after nocturnal GHB challenge.

Conclusions. GHB has postacute effects on peripheral biomarkers of neuropsychiatric disorders, which might be a model to explain some of its therapeutic effects in disorders involving neuro-immunological pathologies (ClinicalTrials.gov: NCT02342366).

Keywords: Gamma-hydroxybutyrate, GHB, TRYCATS, kynurenine pathway, Cortisol, BDNF, neuroinflammation, neuropsychiatric disorders

Significance statement: GHB is used medically and experimentally in neuropsychiatric disorders such as narcolepsy, Parkinson's and Alzheimer's disease, and depression. These disorders involve neuro-immune and neuro-inflammatory processes, especially of the tryptophan catabolites (TRYCATs) pathway. We found that nocturnal GHB reduces next morning's metabolite levels of the kynurenine branch of the TRYCAT pathway and the cortisol awakening response in healthy subjects. This may be a mechanism by which the drug exerts its clinical effects in above mentioned disorders.

Introduction

Gamma-hydroxybutyrate (GHB, or sodium oxybate) is a short-chain fatty acid that occurs naturally in the mammalian brain (Bessman and Fishbein, 1963). It is a gamma-aminobutyric acid (GABA) metabolite that binds as an agonist with high affinity to so-called GHB receptors and with a much lower affinity to $GABA_B$ receptors (Benavides et al., 1982). GHB unfolds unique sleep-augmenting properties, and unlike other sleep medications, it has proven efficacy in the treatment of narcolepsy, a debilitating sleep-regulation disorder (Boscolo-Berto et al., 2012). Its potential to treat other neuropsychiatric disorders associated with insomnia is currently being investigated in patients suffering from Parkinson's (Buchele et al., 2018) and Alzheimer's disease (Klein et al., 2015), fibromyalgia (Spaeth et al., 2012) and depression (Mamelak, 2009; Bosch et al., 2012). Interestingly, insomnia does not only represent a cardinal symptom in those disorders, but is also being discussed as a main driver of pathological alterations in systems engaged with neuro-inflammation, stress processing and neuroplasticity (Maes, 2011; Maurovich-Horvat et al., 2014; Qin et al., 2016; Tsilioni et al., 2016; Decourt et al., 2017). Thus, it has been argued, that GHB may beneficially modulate the disease course, by restoring the homeostatic functions physiological sleep has on those systems (Mamelak, 2009; Bosch et al., 2012). Interestingly, neuro-inflammation, stress processing and neuroplasticity have been found to be substantially related to each other, with inflammation being a key trigger of pathological alterations in the other. Several neuropsychiatric pathologies are characterized by increased levels of pro-inflammatory cytokines, excitotoxicity, oxidative stress and impaired mood (Maes et al., 2011). Thereby, an important downstream effect of increased pro-inflammatory cytokine release is the activation of the kynurenine pathway (KYNP). The KYNP has been proposed as a biochemical hub, linking inflammatory processes, excitotoxicity and impaired mood in neuropsychiatric disorders (Maes et al., 2011). Activation of the KYNP triggers the degradation of tryptophan to partly neurotoxic metabolites, thereby reducing the available amount of serotonin in the brain, a major mood-stabilizing neurotransmitter (Maes et al., 2011). The entry

point of the KYNP are two rate-limiting enzymes: indoleamine 2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO) (Myint and Kim, 2014). IDO is activated by neuro-inflammatory processes, primarily by IFN- γ, but also by TNF-α (Takikawa et al., 1988; Pemberton et al., 1997). On the other hand, TDO is activated by psychophysiological stress via cortisol release (Myint and Kim, 2014). Both enzymes metabolize tryptophan to kynurenine, which is metabolized to kynurenic acid. This metabolic pathway constitutes the neuroprotective branch, because kynurenic acid acts as an antagonist at glutamatergic N-methyl-D-aspartate (NMDA) receptors and reduces glutamate-related neuronal excitotoxicity (Klein et al., 2013). There is also a neurotoxic branch, in which kynurenine is metabolized to 3-hydroxykynurenic acid and quinolinic acid. Both of these metabolites are supposed to be neurotoxic and oxidative stress inducing with depressogenic and anxiogenic effects, and serve as biomarkers for neurodegeneration (Maes et al., 2011).

Moreover, inflammation has been related to enhanced cortisol secretion via the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Bellavance and Rivest, 2014). The cortisol awakening response (CAR) represents a reliable method to assess HPA axis activity (Stalder et al., 2016), and has been shown to be increased in depression (Boggero et al., 2017). Intriguingly, increased HPA axis activity not only triggers the KYNP, but as well reduces levels of the brain derived neurotrophic factor (BDNF) (Masi and Brovedani, 2011), a neurotrophin which promotes neuroplasticity, and has been discussed to substantially contribute to the pathogenesis of above mentioned disorders (Lu et al., 2014; Xu et al., 2015). Indeed, a possible mechanism of action of classical antidepressants may rely partly on the induction of BDNF expression and subsequent increased neuroplasticity (Autry and Monteggia, 2012).

Interestingly, GHB unfolds immunomodulating and neuroprotective properties. In humans, it induces a delayed reduction of T helper and natural killer cell levels – both are activators of pro-inflammatory processes by releasing cytokines such as interferon gamma (IFN- γ) and tumor necrosis factor-alpha (TNF-α) (Pichini et al., 2010). In animals, GHB was shown to protect neuronal tissue against oxidative

stress (Gao et al., 2008). Moreover, GHB appears to have homeostatic regulatory effects on the HPA axis, this is, it increases blunted cortisol levels and reduces enhanced levels (Van Cauter et al., 1997; Nava et al., 2007). Despite its promising effects on neuro-immune processes, it is unknown to date, how a nocturnal dose of GHB affects downstream neuroinflammatory processes, such as the KYNP, BDNF expression, and HPA-axis activity on the next day.

To this end, we here investigate the effects of a sleep-inducing dose of GHB (50 mg/kg p.o) vs. placebo on morning serum levels of tryptophan catabolites (TRYCATs) including kynurenic acid, kynurenine, xanthurenic acid, 3-hydroxy-kynurenine, quinolinic acid, serotonin, and tryptophan and as well BDNF levels and the CAR in twenty healthy male volunteers. Additionally, the Positive and Negative Affect Schedule (PANAS) was applied to assess potential effects on mood. We used a postacute assessment, because the immunomodulating effects of GHB have been reported to start with a delay of 2-4 hours (Pichini et al., 2010), and nocturnal dosing followed by therapeutic effects during daytime is the standard pattern in the treatment of narcolepsy (Boscolo-Berto et al., 2012). The study followed a placebo-controlled, balanced, randomized, double-blind, cross-over design. In conclusion of the above mentioned effects of GHB on blood biomarkers of neuropsychiatric disorders, we expected that the drug would reduce KYNP metabolite levels and the CAR, while increasing serotonin, tryptophan, and BDNF levels and enhance mood.

Materials and Methods

Permission

The study was approved by the Swissmedic and Cantonal Ethics Committee of Zurich and registered at ClinicalTrials.gov (NCT02342366). All participants provided written informed consent according to the declaration of Helsinki.

Participants

Twenty healthy, non-smoking, Caucasian, male students (mean age 25.8±2.45 years) participated in the study. Following criteria were required for inclusion: male sex to avoid the potential impact of menstrual cycle on blood chemical variables, age within the range of 18 to 30 years, absence of any somatic or psychiatric disorders, no first-degree relatives with a history of psychiatric disorders, non-smoking, without a history of drug abuse (lifetime use > 5 occasions, with exception of occasional cannabis use). None of the participants reported previous experiences with GHB in their life. Before inclusion into the study, each subject was screened with all-night polysomnography in the sleep laboratory, to exclude sleep-related disorders such as sleep apnea, restless leg syndrome, sleep onset REM sleep, and insufficient sleep efficiency (< 80%). All participants were recruited via advertisements on the online student job platform of the University of Zurich. All subjects were monetary compensated for the completion of the study.

Study design

The study followed a placebo-controlled, randomized, balanced, cross-over design. Two experimental nights were separated by a washout phase of seven days. Each experimental night was preceded by an adaptation night to habituate subjects to the laboratory environment.

Figure 1

Participants were instructed to refrain from illegal drugs for at least two weeks and from caffeine for

one week prior to the first and until the second experimental night. No alcohol was allowed 24 h before each experimental night. Participants had to keep a regular sleep-wake rhythm with a bedtime of eight hours from 11:00 p.m. to 07:00 a.m. for one week prior to the first experimental night and during the week between the two experimental nights. To ensure adherence to these instructions, participants wore an actimeter on the non-dominant arm and kept a sleep-wake diary. All participants received a monetary compensation for the completion of the study.

Drug administration

GHB is currently the first-line, FDA-approved treatment for cataplexy and excessive daytime sleepiness in patients with narcolepsy (Boscolo-Berto et al., 2012). GHB-induced neuro-immunological alterations were previously shown to start at 2-4 hours after ingestion (Pichini et al., 2010). The drug is typically administered twice, an initial dose at bedtime and a second dose 2.5-4 hours later, to stabilize the severely disturbed sleep continuity in narcolepsy patients (Boscolo-Berto et al., 2012). Therfore, a single oral dose of GHB was administered 3.5 hours after bedtime. All study participants were awoken at 02:30 am and received 50 mg/kg of GHB dissolved in 2 dl of orange juice and a placebo, matched in appearance and taste. Immediately after GHB ingestion, subjects were allowed to return to sleep. The used dose represents the starting dose used for the treatment of narcolepsy (Boscolo-Berto et al., 2012).

Urine Drug Screening

Urine samples were taken upon arrival in order to ensure that all participants abstained from illegal drug use (Drug-Screen Multi 12-AE, Nal von Minden GmbH, Regensburg, DE). Additional urine samples were collected in the morning following experimental nights to confirm GHB ingestion. Morning urine samples were analyzed with a cloned enzyme donor immunoassay (CEDIA®, Indiko plus, Thermo Fisher Scientific, San Jose, USA) for GHB with a cut-off concentration of 40 mg/L for GHB.

Blood collection

Venous blood was sampled from the ante-cubital vein at 10:00 a.m. After a coagulation time of 30 minutes, blood was centrifuged for 10 minutes at 2000 RCF to obtain clear serum. Supernatant serum was then distributed to Eppendorf tubes and immediately stored at -80°C.

Tryptophan catabolites

TRYCATs were analyzed using a ultra-high performance liquid chromatography (UHPLC) system (Thermo Fisher, San Jose, California, USA) coupled to a 5500 linear ion trap quadrupole mass spectrometer (Sciex, Darmstadt/Germany). The method was validated according to the guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh) (Peters et al., 2007). For details, see supplementary material.

Cortisol awakening response

Saliva of each subject was sampled at time points 7:00 (immediately after awakening), 7:15, 7:30, 7:45, and 8:00 a.m. Thereby, subjects were instructed to chew the swab for 60 seconds and then return it in to the Salivette® tube (Sarstedt, Germany). After sampling, tubes were immediately stored on ice until final storage at -80°C. For cortisol detection, tubes were defrosted and centrifuged for 5 minutes at 5'000 rpm to yield clear saliva in the conical tube. Three subjects had to be excluded, as the amount of saliva yielded from the swabs was insufficient for further analysis. Then, the swab was removed and the yielded saliva was used for further analysis. A liquid-liquid extraction was carried out by adding 1.5 mL ethyl acetate to 265 µL of saliva sample and 50 µl IS (Cortison-D_7 0.1 ng/µL). The resulting mixture was subsequently shaken for 10 min at 5 Hz. The samples were centrifuged for 5 min at 9000 rpm and then placed in a freezer (-20° C) for approximately 60 min. The ethyl acetate layer was poured off and dried under nitrogen at 35° C. The dry residue was re-suspended using 150 µL MeOH and 350 µL ammonium formate (5 mM) solution which was used for

liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis following a recently published method using $^{13}C_3$-labeled cortisol as surrogate analyte for calibration (Binz et al., 2016). The method was validated according to the guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh) (Peters et al., 2007). The limit of detection for cortisol was 0.55 nmol/L and the limit of quantification was 1.1 nmol/L.

Brain derived neurotrophic factor

Quantification of serum BDNF levels was conducted at the Department of Clinical Psychology and Psychotherapy at the University of Zurich using a 96-Well MULTI-ARRAY® BDNF Assay (Meso-Scale Discovery, Rockville, Maryland). The analysis was performed according to the manufacturer's instruction.

Positive and Negative Affect Schedule

Post-awakening mood was assessed at 10 a.m. using the PANAS, a questionnaire in which the participant is asked to rate the occurrence and intensity of 20 mood states (ten positive, ten negative adjectives) on a 5-point Likert scale in the moment of the rating (Watson et al., 1988).

Statistical analysis

All analyses were conducted using RStudio Version 1.0.136 (RStudio, Inc.). Three independent Linear Mixed Effects Models (LME) were used for TRYCATS, CAR, and BDNF levels. Posthoc testing was carried out using the R package *emmeans* (Version 1.2.1). Individual averages for each log-transformed TRYCAT level and the 3-hydroxykynurenine to kynurenic acid and the quinolinic acid to kynurenic acid ratios were entered in a LME, whereas possible factors were *condition* (GHB vs. placebo) and *metabolite* (type of TRYCAT). Furthermore, statistical differences ($p<0.05$) of each TRYCAT was tested using post-hoc t-tests, applying Benjamini-Hochberg correction to adjust for multiple comparison (Hochberg and Benjamini, 1990). Individual log-transformed cortisol levels were

entered in a LME, whereas possible factors were *condition* (GHB vs. placebo) and *time point* (7:00, 7:15, 7:30, 7:45, 8:00 a.m.). Furthermore, statistical differences ($p<0.05$) in cortisol levels at each time point were tested using post-hoc t-tests, applying Benjamini-Hochberg correction to adjust for multiple comparison. Individual log-transformed BDNF levels were entered in a LME, with *condition* (GHB vs. placebo) as possible factors. Furthermore, statistical differences ($p<0.05$) in BDNF levels were tested using post-hoc t-tests, applying Benjamini-Hochberg correction to adjust for multiple comparison.

For all applied models, normal Q-Q plots were applied, demonstrating normality of the residuals. Moreover, the assumption of homoscedasticity and linearity was verified using a Tukey-Anscombe (residuals vs fitted) plot.

Results

Urine Drug Screening

Drug screening revealed no positive test results for all subjects on both experimental nights. Morning urine analysis revealed a positive result for GHB exclusively in the drug condition of all investigated subjects.

Tryptophan catabolites

Statistical analysis revealed a significant main effect for condition ($F(1,475)=28.81$; $p<0.001$; $\eta^2=0.057$). Posthoc tests further revealed reduced levels of indolelactic acid ($p<0.01$), kynurenine ($p<0.05$), kynurenic acid ($p<0.01$), 3-hydroxykynurenine ($p<0.001$), Quinolinic acid ($p<0.001$) and the 3-hydroxykynurenine to kynurenic acid ratio ($p<0.01$) in the GHB condition compared to placebo (corrected for multiple comparisons). Tryptophan and the quinolinic acid to kynurenic acid ratio were reduced on a trend level (both $p=0.085$). All other metabolites remained unaffected by the drug. Results of statistical analysis are summarized in Table 1.

Tryptophan catabolites levels (nM) and ratios

	Placebo		GHB				
Metabolites	Mean	SD	Mean	SD	t-value	Cohen's d	p-value
Indolelactic acid	1100	180	990	160	-3.37	1.18	< 0.01 **
Serotonin	1000	370	940	370	-1.78	1.54	0.11
Kynurenic acid	61	4.9	58	2.9	-4.28	0.75	< 0.01 **
Tryptophan	90000	13000	85000	8700	-2.05	0.75	0.085
Kynurenine	2600	390	2400	380	-2.90	0.78	< 0.05 *
Xanthurenic acid	25	7.9	36	49	0.97	-1.44	0.37
3-Hydroxykynurenine	35	6.7	30	5.4	-6.00	1.53	< 0.001 ***
Hydroxyindoleacetic acid	39	8.2	38	5.1	-0.86	0.65	0.40
Quinolinic acid	450	100	410	97	-5.22	1.35	< 0.001 ***
3-HK:KYNA ratio	0.57	0.09	0.53	0.09	-3.33	0.77	< 0.01 **
QUIN:KYNA ratio	7.4	1.6	7.0	1.7	-2.00	0.60	0.085

Figure 2

Cortisol awakening response

Statistical analysis revealed a significant main effect for condition ($F(1, 125.41)=4.08$; $p<0.05$; $\eta^2=0.029$). Posthoc testing further revealed reduced cortisol levels at time points 7:00, 7:15 and 7:30 a.m. in the GHB condition compared to placebo (corrected for multiple comparisons). By contrast, cortisol levels at time points 7:45 and 8:00 a.m. remained unaffected by the drug.

Figure 3

Brain derived neurotrophic factor

Statistical analysis revealed no significant different BDNF levels between the GHB and the placebo condition ($F(1,18.07)=0.29$; $p=0.59$; $\eta^2=0.01$).

Figure 4

Positive and Negative Affect Schedule

Statistical analysis revealed no significant differences in the PANAS rating between the GHB and the placebo condition ($F(1,56.99)=0.02$; $p=0.87$; $\eta^2=0.0004$).

Discussion

Here, we investigated the effects of the mixed GHB-/$GABA_B$ receptor agonist GHB on peripheral biomarkers of neuropsychiatric disorders, assessing serum levels of TRYCATs and BDNF, as well as the CAR from saliva and subjective mood effects using the PANAS. We found that in healthy male subjects, a nocturnal dose of 50 mg/kg p.o. GHB reduces peripheral levels of the KYNP metabolites indolelactic acid, kynurenic acid, kynurenine, 3-hydroxykynurenine, and quinolinic acid 7.5 h after GHB intake, while BDNF, serotonin and tryptophan levels as well as PANAS scores remained unchanged. Moreover, GHB reduced the CAR 4,5 h after drug intake.

Sleep disturbances represent a cardinal symptom in several neuropsychiatric disorders (Khurshid, 2018) and may represent a main driver of pathological alterations in systems engaged with neuro-inflammation, stress processing and neuroplasticity (Krysta et al., 2017; Irwin and Piber, 2018; Irwin and Vitiello, 2019)). GHB is an internationally established treatment of narcolepsy, but is also experimentally used and investigated to treat Parkinson's (Buchele et al., 2018) and Alzheimer's disease (Klein et al., 2015), as well as depression (Mamelak, 2009; Bosch et al., 2012). The therapeutic efficiency of GHB in these disorders is classically attributed to its unique sleep consolidating effects, as evening/nocturnal doses of the drug strongly increase deep sleep and reduce daytime sleepiness and fatigue during the next day (Van Cauter et al., 1997; Buchele et al., 2018; Dornbierer et al., 2019). Given the important relationship between sleep and neuro-immunological functioning, it has been argued, that GHB may beneficially modulate neuro-inflammation and its downstream effects, by restoring sleep's regenerating functions on the immune system (Gao et al., 2008; Mamelak, 2009; Klein et al., 2015).

An important pro-inflammatory downstream effect of increased cytokine release is the activation of the KYNP and has been proposed as biochemical hub, linking inflammatory processes and impaired mood in neuropsychiatric disorders (Anderson and Maes, 2014; Maes and Anderson, 2016; Ogyu et al., 2018). The metabolization of tryptophan down the KYNP is initiated by the enzymes IDO and

TDO, thereby reducing neuronal levels of tryptophan, serotonin, and melatonin. IDO is strongly activated by inflammatory cytokines such as IFN-γ and TNF-α, and inhibition of this rate-limiting enzyme prevents the development of inflammation-induced depression- and anxiety-like behaviors (O'Connor et al., 2009; Salazar et al., 2012). Moreover, the products of this process are partly neurotoxic and pro-inflammatory, such as 3-hydroxykynurenine and quinolinic acid (Chiarugi et al., 2001). Intriguingly, in our study, GHB induced an inhibition of the putatively neurotoxic KYNP metabolites 3-hydroxykynurenine and quinolinic acid, but also of kynurenine and the putative neuroprotective kynurenic acid. Intriguingly, this effect outlived the acute drug phase, given the short half-life of GHB (30-50 min) and the late time point of blood sampling ($t_{7.5h}$). Inhibition of the KYNP by GHB may occur on several levels, but most evidences point towards indirect interactions of the drug with the function of the two rate-limiting enzymes of tryptophan degradation, IDO and TDO. First, GHB was found to induce a delayed reduction of T helper and natural killer cell levels in humans (Pichini et al., 2010). These lymphocytes are major cytokine producers and it is likely that GHB reduces the plasma cytokine load via this mechanism. Second, direct $GABA_B$ receptor stimulation of astrocytes (Gould et al., 2014) and microglia (Kuhn et al., 2004) were likewise found to reduce the release of the pro-inflammatory cytokines TNF-α and IL-6, both of which were identified as the most reliable inflammatory biomarkers of major depression (Miller and Raison, 2016). The IDO gene is predominantly activated by IFN-γ, whereas TNF-α synergistically increases the transcriptional stimulation of IDO synthesis in response to IFN-γ by up to 300% (Robinson et al., 2005). A third mechanism, by which GHB might indirectly inhibit IDO activity, is via increased progesterone release (Bosch et al., 2015). Progesterone attenuates IFN- γ-mediated KYNP metabolite synthesis in human macrophages *in vitro*, leading to decreased levels of quinolinic acid and increased kynurenic acid levels (de Bie et al., 2016). Thus, GHB-induced lymphocyte reduction and astrocyte activation might reduce cytokine levels and thus IDO activity, while progesterone release seems to directly inhibit neurotoxic KYNP metabolite formation in macrophages.

The other rate-limiting enzyme of the KYNP is TDO. Reduction of TDO activity was early hypothesized to be an antidepressant treatment mechanism (Lapin and Oxenkrug, 1969), as TDO degrades approximately 99% of tryptophan in the periphery (Watanabe et al., 1980). Although TDO activity is generally stable and mainly controlled by the tryptophan level itself, stress-related cortisol release can enhance TDO degradation of tryptophan (Nakamura et al., 1987). The GHB-induced CAR reduction found in our study, indicates a down-regulation of HPA axis activity and may be another mechanism by which the drug inhibits the KYNP. In fact, enhancement of IDO activity by cytokines and enhancement of TDO activity by cortisol are hypothesized to be the core mechanisms of KYNP metabolite mediated degenerative neuropsychiatric disorders (Myint and Kim, 2014), and both seem to be inhibited by GHB in humans.

The two putatively neurotoxic products of the KYNP are 3-hydroxykynurenine and quinolinic acid. The serum levels of both molecules were reduced the next morning after GHB ingestion in our subjects. 3-hydroxykynurenine is a free radicals producer potentially involved in the mediation of oxidative stress and neuronal cell death (Chiarugi et al., 2001). Furthermore, 3-hydroxykynurenine can cause oxidative protein damage by generating superoxide and hydrogen peroxide (Goldstein et al., 2000). Several studies found associations of 3-hydroxykynurenine with neuropsychiatric and neurodegenerative pathologies: Memory deficits were associated with the molecule in patients with bipolar (Platzer et al., 2017) and major depressive disorder (Young et al., 2016). One study with 20 patients suffering from Alzheimer's disease revealed elevated 3-hydroxykynurenine plasma levels compared to controls (Schwarz et al., 2013). Another study with 48 patients with Parkinson's disease, found an increase of 3-hydroxykynurenine by 30% in a metabolomics analysis of cerebrospinal fluid (Lewitt et al., 2013).

The other potentially neurotoxic KYNP metabolite, quinolinic acid, acts as an agonist at NR2A and NR2B subtypes of glutamatergic NMDA receptors and thereby mediates excitotoxicity (de Carvalho et al., 1996). It is also a free radicals producer, as it induces nitric oxide synthase and excessive nitric oxide-mediated free radical damage (Braidy et al., 2009). As with 3-hydroxykynurenine, elevated

quinolinic acid (Guillemin et al., 2005) and kynurenic acid (Jacobs et al., 2019) levels were associated with Alzheimer's disease, Parkinson's disease (Nemeth et al., 2006), and depression (Ogyu et al., 2018).

The pathophysiological role of indolelactic acid, which was also reduced by GHB in our participants, is still unclear. Some evidences point towards increased indolelactic acid in liver toxicity (Manna et al., 2010).

Not only neurotoxic KYNP metabolites such as quinolinic acid and 3-hydroxykynurenine, but also the putatively neuroprotective KYNP metabolite kynurenic acid was reduced after GHB administration in our participants. This was most likely due to an inhibition on the IDO and TDO activity, as all three molecules are downstream metabolites of these enzymes. However, it seems that this inhibition is stronger on the neurotoxic KYNP branch, as GHB also reduced the 3-hydroxykynurenine to kynurenic acid ratio significantly and the quinolinic acid to kynurenic acid ratio by trend. A comparable effect was already shown after electroconvulsive therapy (Guloksuz et al., 2015) and after antidepressant medication (Kocki et al., 2012) in depression.

Anti-oxidative effects of GHB are well-established, as the drug limits the damage caused by reactive oxygen species, lipid peroxides, and other reactive molecules (for review see (Mamelak, 2007)). However, reduction of oxidative stress via inhibition of 3-hydroxykynurenine and/or quinolinic acid has not been documented to date. It was shown that $GABA_B$ agonism reduces excitatory glutamatergic neurotransmission in the brain (Chalifoux and Carter, 2011). So, GHB seems to inhibit a major source of neuroinflammation-induced excitotoxicity and via $GABA_B$ agonism also the target receptor of excitotoxic molecules.

Further evidence for a modulation of human neuroendocrine and neuro-immune pathways by GHB is given by our finding that the drug reduces saliva CAR. The stress hormone cortisol is the end product of the HPA axis, and the CAR reflects the steep increase of cortisol occurring immediately after awakening. Diverse factors such as psychological functioning, psychiatric disorders, and alterations of the sleep-wake cycle have an impact on the CAR, but there is considerable heterogeneity in the

literature (Elder et al., 2014). However, evidences from meta-analyses point to associations of depression, stress and elevated vigilance with high and history of posttraumatic stress, fatigue, as well as schizophrenia with low cortisol output (Chida and Steptoe, 2009; Boggero et al., 2017). Consequently, GHB-induced CAR reduction may be interpreted in line with potential antidepressant and stress-reducing effects of the drug.

Another disease relevant biomarker, which has been found to be affected by increased inflammation and psychophysiological stress is BDNF (Masi and Brovedani, 2011; Calabrese et al., 2014). It is a neurotrophin which promotes neuroplasticity, and has been discussed to substantially contribute to the pathogenesis of neuropsychiatric disorders (Lu et al., 2014; Xu et al., 2015). Despite the modulatory effects of GHB on the KYNP and CAR observed in this study, we did not find any drug effects on BDNF levels. Several reasons may account for this, such that changes in BDNF levels rely on the induction/inhibition of the BDNF gene expression, which may take several days or weeks to occur, as it is the case for antidepressants (Autry and Monteggia, 2012). Moreover, it is not fully understood how peripheral blood levels of BDNF related to its concentration the central nervous system.

Subjective mood was assessed using the PANAS, but no significant postacute drug effect was found. As we tested the drug in healthy participants, a potential antidepressant effect was not addressed. However, overall tolerability of GHB was good, and no severe adverse effects occurred.

Anyhow, our study has limitations. First, it was performed with healthy volunteers and not in a clinical sample. Therefore, further studies with patients suffering from neuropsychiatric disorders involving neuro-immune pathologies should be performed to test if GHB induces the same effects on TRYCATs and CAR along with therapeutic effects in these subjects. Second, we only applied a single dose of GHB instead of a continuous treatment as it is usually used in narcolepsy patients. Studies with repeated dosing should evaluate the course of blood levels of TRYCATs and BDNF and the CAR during GHB treatment in healthy participants and patient populations.

In summary, in healthy volunteers, a single nocturnal dose of GHB significantly reduces post-awakening serum levels of several KYNP metabolites, including indolelactic acid, kynurenine, kynurenic acid, 3-hydroxykynurenine, quinolinic acid, as well as the 3-hydroxykynurenine to kynurenic acid ratio. Moreover, CAR was significantly reduced. These effects are most likely mediated by indirect inhibition of the rate-limiting enzymes of the KYNP, IDO and TDO. IDO may be inhibited via multiple mechanisms that reduce the serum cytokine load, while TDO seems to be inhibited via reduction of HPA axis activity. These effects show that GHB modulates neuro-inflammatory and neuro-immune pathways in humans and suggest that this mechanism may be responsible for its existing and potential therapeutic effects in neuropsychiatric and neurodegenerative disorders including narcolepsy, depression, as well as Alzheimer's and Parkinson's disease.

Acknowledgments:

The authors thank Vinnie Kandra for her dedicated assistance in data collection and participant recruitment.

Funding and disclosure:

All authors declare no conflict of interest.

References

Anderson G, Maes M (2014) TRYCAT pathways link peripheral inflammation, nicotine, somatization and depression in the etiology and course of Parkinson's disease. CNS Neurol Disord Drug Targets 13:137-149.

Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64:238-258.

Barbaccia ML (2004) Neurosteroidogenesis: relevance to neurosteroid actions in brain and modulation by psychotropic drugs. Crit Rev Neurobiol 16:67-74.

Bellavance MA, Rivest S (2014) The HPA - Immune Axis and the Immunomodulatory Actions of Glucocorticoids in the Brain. Front Immunol 5:136.

Benavides J, Rumigny JF, Bourguignon JJ, Cash C, Wermuth CG, Mandel P, Vincendon G, Maitre M (1982) High affinity binding sites for gamma-hydroxybutyric acid in rat brain. Life sciences 30:953-961.

Bessman SP, Fishbein WN (1963) Gamma-Hydroxybutyrate, a Normal Brain Metabolite. Nature 200:1207-1208.

Binz TM, Braun U, Baumgartner MR, Kraemer T (2016) Development of an LC-MS/MS method for the determination of endogenous cortisol in hair using (13)C3-labeled cortisol as surrogate analyte. J Chromatogr B Analyt Technol Biomed Life Sci 1033-1034:65-72.

Boggero IA, Hostinar CE, Haak EA, Murphy MLM, Segerstrom SC (2017) Psychosocial functioning and the cortisol awakening response: Meta-analysis, P-curve analysis, and evaluation of the evidential value in existing studies. Biol Psychol 129:207-230.

Bosch OG, Quednow BB, Seifritz E, Wetter TC (2012) Reconsidering GHB: orphan drug or new model antidepressant? Journal of psychopharmacology 26:618-628.

Bosch OG, Eisenegger C, Gertsch J, von Rotz R, Dornbierer D, Gachet MS, Heinrichs M, Wetter TC, Seifritz E, Quednow BB (2015) Gamma-hydroxybutyrate enhances mood and prosocial behavior without affecting plasma oxytocin and testosterone. Psychoneuroendocrinology 62:1-10.

Boscolo-Berto R, Viel G, Montagnese S, Raduazzo DI, Ferrara SD, Dauvilliers Y (2012) Narcolepsy and effectiveness of gamma-hydroxybutyrate (GHB): a systematic review and meta-analysis of randomized controlled trials. Sleep medicine reviews 16:431-443.

Braidy N, Grant R, Adams S, Brew BJ, Guillemin GJ (2009) Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. Neurotox Res 16:77-86.

Buchele F, Hackius M, Schreglmann SR, Omlor W, Werth E, Maric A, Imbach LL, Hagele-Link S, Waldvogel D, Baumann CR (2018) Sodium Oxybate for Excessive Daytime Sleepiness and Sleep Disturbance in Parkinson Disease: A Randomized Clinical Trial. JAMA Neurol 75:114-118.

Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R (2014) Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front Cell Neurosci 8:430.

Carter LP, Koek W, France CP (2009) Behavioral analyses of GHB: receptor mechanisms. Pharmacology & therapeutics 121:100-114.

Chalifoux JR, Carter AG (2011) GABAB receptor modulation of synaptic function. Curr Opin Neurobiol 21:339-344.

Chiarugi A, Meli E, Moroni F (2001) Similarities and differences in the neuronal death processes activated by 3OH-kynurenine and quinolinic acid. J Neurochem 77:1310-1318.

Chida Y, Steptoe A (2009) Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol Psychol 80:265-278.

Cui W, Ning Y, Hong W, Wang J, Liu Z, Li MD (2018) Crosstalk Between Inflammation and Glutamate System in Depression: Signaling Pathway and Molecular Biomarkers for Ketamine's Antidepressant Effect. Mol Neurobiol.

de Bie J, Lim CK, Guillemin GJ (2016) Progesterone Alters Kynurenine Pathway Activation in IFN-gamma-Activated Macrophages - Relevance for Neuroinflammatory Diseases. Int J Tryptophan Res 9:89-93.

de Carvalho LP, Bochet P, Rossier J (1996) The endogenous agonist quinolinic acid and the non endogenous homoquinolinic acid discriminate between NMDAR2 receptor subunits. Neurochem Int 28:445-452.
Decourt B, Lahiri DK, Sabbagh MN (2017) Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. Curr Alzheimer Res 14:412-425.
Dornbierer DA, Baur DM, Stucky B, Quednow BB, Kraemer T, Seifritz E, Bosch OG, Landolt HP (2019) Neurophysiological signature of gamma-hydroxybutyrate augmented sleep in male healthy volunteers may reflect biomimetic sleep enhancement: a randomized controlled trial. Neuropsychopharmacology.
Elder GJ, Wetherell MA, Barclay NL, Ellis JG (2014) The cortisol awakening response--applications and implications for sleep medicine. Sleep medicine reviews 18:215-224.
Gao B, Kilic E, Baumann CR, Hermann DM, Bassetti CL (2008) Gamma-hydroxybutyrate accelerates functional recovery after focal cerebral ischemia. Cerebrovasc Dis 26:413-419.
Goldstein LE, Leopold MC, Huang X, Atwood CS, Saunders AJ, Hartshorn M, Lim JT, Faget KY, Muffat JA, Scarpa RC, Chylack LT, Jr., Bowden EF, Tanzi RE, Bush AI (2000) 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen peroxide and promote alpha-crystallin cross-linking by metal ion reduction. Biochemistry 39:7266-7275.
Gould T, Chen L, Emri Z, Pirttimaki T, Errington AC, Crunelli V, Parri HR (2014) GABA(B) receptor-mediated activation of astrocytes by gamma-hydroxybutyric acid. Philos Trans R Soc Lond B Biol Sci 369:20130607.
Guillemin GJ, Brew BJ, Noonan CE, Takikawa O, Cullen KM (2005) Indoleamine 2,3 dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. Neuropathol Appl Neurobiol 31:395-404.
Guloksuz S, Arts B, Walter S, Drukker M, Rodriguez L, Myint AM, Schwarz MJ, Ponds R, van Os J, Kenis G, Rutten BP (2015) The impact of electroconvulsive therapy on the tryptophan-kynurenine metabolic pathway. Brain Behav Immun 48:48-52.
Hochberg Y, Benjamini Y (1990) More powerful procedures for multiple significance testing. Stat Med 9:811-818.
Irwin MR, Piber D (2018) Insomnia and inflammation: a two hit model of depression risk and prevention. World Psychiatry 17:359-361.
Irwin MR, Vitiello MV (2019) Implications of sleep disturbance and inflammation for Alzheimer's disease dementia. Lancet Neurol 18:296-306.
Jacobs KR, Lim CK, Blennow K, Zetterberg H, Chatterjee P, Martins RN, Brew BJ, Guillemin GJ, Lovejoy DB (2019) Correlation between plasma and CSF concentrations of kynurenine pathway metabolites in Alzheimer's disease and relationship to amyloid-beta and tau. Neurobiol Aging 80:11-20.
Khurshid KA (2018) Comorbid Insomnia and Psychiatric Disorders: An Update. Innov Clin Neurosci 15:28-32.
Klein C, Mathis C, Leva G, Patte-Mensah C, Cassel JC, Maitre M, Mensah-Nyagan AG (2015) gamma-Hydroxybutyrate (Xyrem) ameliorates clinical symptoms and neuropathology in a mouse model of Alzheimer's disease. Neurobiol Aging 36:832-844.
Klein C, Patte-Mensah C, Taleb O, Bourguignon JJ, Schmitt M, Bihel F, Maitre M, Mensah-Nyagan AG (2013) The neuroprotector kynurenic acid increases neuronal cell survival through neprilysin induction. Neuropharmacology 70:254-260.
Kocki T, Wnuk S, Kloc R, Kocki J, Owe-Larsson B, Urbanska EM (2012) New insight into the antidepressants action: modulation of kynurenine pathway by increasing the kynurenic acid/3-hydroxykynurenine ratio. J Neural Transm (Vienna) 119:235-243.
Krysta K, Krzystanek M, Bratek A, Krupka-Matuszczyk I (2017) Sleep and inflammatory markers in different psychiatric disorders. J Neural Transm (Vienna) 124:179-186.
Kuhn SA, van Landeghem FK, Zacharias R, Farber K, Rappert A, Pavlovic S, Hoffmann A, Nolte C, Kettenmann H (2004) Microglia express GABA(B) receptors to modulate interleukin release. Mol Cell Neurosci 25:312-322.

Lapin IP, Oxenkrug GF (1969) Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet 1:132-136.
Lewitt PA, Li J, Lu M, Beach TG, Adler CH, Guo L, Arizona Parkinson's Disease C (2013) 3-hydroxykynurenine and other Parkinson's disease biomarkers discovered by metabolomic analysis. Mov Disord 28:1653-1660.
Lu B, Nagappan G, Lu Y (2014) BDNF and synaptic plasticity, cognitive function, and dysfunction. Handb Exp Pharmacol 220:223-250.
Maes M (2011) Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression. Prog Neuropsychopharmacol Biol Psychiatry 35:664-675.
Maes M, Anderson G (2016) Overlapping the Tryptophan Catabolite (TRYCAT) and Melatoninergic Pathways in Alzheimer's Disease. Curr Pharm Des 22:1074-1085.
Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R (2011) The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry 35:702-721.
Mamelak M (2007) Alzheimer' s disease, oxidative stress and gammahydroxybutyrate. Neurobiol Aging 28:1340-1360.
Mamelak M (2009) Narcolepsy and depression and the neurobiology of gammahydroxybutyrate. Progress in neurobiology 89:193-219.
Manna SK, Patterson AD, Yang Q, Krausz KW, Li H, Idle JR, Fornace AJ, Jr., Gonzalez FJ (2010) Identification of noninvasive biomarkers for alcohol-induced liver disease using urinary metabolomics and the Ppara-null mouse. J Proteome Res 9:4176-4188.
Masi G, Brovedani P (2011) The hippocampus, neurotrophic factors and depression: possible implications for the pharmacotherapy of depression. CNS Drugs 25:913-931.
Maurovich-Horvat E, Keckeis M, Lattova Z, Kemlink D, Wetter TC, Schuld A, Sonka K, Pollmacher T (2014) Hypothalamo-pituitary-adrenal axis, glucose metabolism and TNF-alpha in narcolepsy. J Sleep Res 23:425-431.
Miller AH, Raison CL (2016) The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16:22-34.
Myint AM, Kim YK (2014) Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis. Prog Neuropsychopharmacol Biol Psychiatry 48:304-313.
Nakamura T, Niimi S, Nawa K, Noda C, Ichihara A, Takagi Y, Anai M, Sakaki Y (1987) Multihormonal regulation of transcription of the tryptophan 2,3-dioxygenase gene in primary cultures of adult rat hepatocytes with special reference to the presence of a transcriptional protein mediating the action of glucocorticoids. J Biol Chem 262:727-733.
Nava F, Premi S, Manzato E, Campagnola W, Lucchini A, Gessa GL (2007) Gamma-hydroxybutyrate reduces both withdrawal syndrome and hypercortisolism in severe abstinent alcoholics: an open study vs. diazepam. Am J Drug Alcohol Abuse 33:379-392.
Nemeth H, Toldi J, Vecsei L (2006) Kynurenines, Parkinson's disease and other neurodegenerative disorders: preclinical and clinical studies. J Neural Transm Suppl:285-304.
O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, Kelley KW, Dantzer R (2009) Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. Mol Psychiatry 14:511-522.
Ogyu K, Kubo K, Noda Y, Iwata Y, Tsugawa S, Omura Y, Wada M, Tarumi R, Plitman E, Moriguchi S, Miyazaki T, Uchida H, Graff-Guerrero A, Mimura M, Nakajima S (2018) Kynurenine pathway in depression: A systematic review and meta-analysis. Neurosci Biobehav Rev 90:16-25.
Pemberton LA, Kerr SJ, Smythe G, Brew BJ (1997) Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. J Interferon Cytokine Res 17:589-595.
Peters FT, Hartung M, Herbold M, Schmitt G, Daldrup T, Musshoff F (2007) Anhang B zur Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Anforderungen an die Validierung vonAnalysenmethoden, . Toxichem Krimtech 76:185.

Pichini S, Farre M, Abanades S, Pacifici R, Zuccaro P, Langohr K, de la Torre R (2010) Immunomodulating properties of gamma-hydroxybutyrate (GHB), flunitrazepam and ethanol in 'club drugs' users. Addict Biol 15:336-345.
Platzer M et al. (2017) Tryptophan breakdown and cognition in bipolar disorder. Psychoneuroendocrinology 81:144-150.
Qin XY, Zhang SP, Cao C, Loh YP, Cheng Y (2016) Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: A Systematic Review and Meta-analysis. JAMA Neurol 73:1316-1324.
Robinson CM, Hale PT, Carlin JM (2005) The role of IFN-gamma and TNF-alpha-responsive regulatory elements in the synergistic induction of indoleamine dioxygenase. J Interferon Cytokine Res 25:20-30.
Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'Connor JC (2012) Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. Horm Behav 62:202-209.
Schwarz MJ, Guillemin GJ, Teipel SJ, Buerger K, Hampel H (2013) Increased 3-hydroxykynurenine serum concentrations differentiate Alzheimer's disease patients from controls. Eur Arch Psychiatry Clin Neurosci 263:345-352.
Smith JR, Jamie JF, Guillemin GJ (2016) Kynurenine-3-monooxygenase: a review of structure, mechanism, and inhibitors. Drug Discov Today 21:315-324.
Spaeth M, Bennett RM, Benson BA, Wang YG, Lai C, Choy EH (2012) Sodium oxybate therapy provides multidimensional improvement in fibromyalgia: results of an international phase 3 trial. Annals of the rheumatic diseases 71:935-942.
Stalder T, Kirschbaum C, Kudielka BM, Adam EK, Pruessner JC, Wust S, Dockray S, Smyth N, Evans P, Hellhammer DH, Miller R, Wetherell MA, Lupien SJ, Clow A (2016) Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinology 63:414-432.
Takikawa O, Kuroiwa T, Yamazaki F, Kido R (1988) Mechanism of interferon-gamma action. Characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-gamma and evaluation of the enzyme-mediated tryptophan degradation in its anticellular activity. J Biol Chem 263:2041-2048.
Tsilioni I, Russell IJ, Stewart JM, Gleason RM, Theoharides TC (2016) Neuropeptides CRH, SP, HK-1, and Inflammatory Cytokines IL-6 and TNF Are Increased in Serum of Patients with Fibromyalgia Syndrome, Implicating Mast Cells. J Pharmacol Exp Ther 356:664-672.
Van Cauter E, Plat L, Scharf MB, Leproult R, Cespedes S, L'Hermite-Baleriaux M, Copinschi G (1997) Simultaneous stimulation of slow-wave sleep and growth hormone secretion by gamma-hydroxybutyrate in normal young Men. J Clin Invest 100:745-753.
Watanabe Y, Fujiwara M, Yoshida R, Hayaishi O (1980) Stereospecificity of hepatic L-tryptophan 2,3-dioxygenase. Biochem J 189:393-405.
Watson D, Clark LA, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 54:1063-1070.
Xu H, Czerwinski P, Xia N, Forstermann U, Li H (2015) Downregulation of BDNF Expression by PKC and by TNF-alpha in Human Endothelial Cells. Pharmacology 96:1-10.
Young KD, Drevets WC, Dantzer R, Teague TK, Bodurka J, Savitz J (2016) Kynurenine pathway metabolites are associated with hippocampal activity during autobiographical memory recall in patients with depression. Brain Behav Immun 56:335-342.

Figure legends:

Figure 1: Session design.

Figure 2: TRYCATs serum concentrations at time point 3h post-awakening/ 7.5 h post-GHB challenge placebo vs. GHB. 3HK: hydroxykynurenine, KYNA: Kynurenic acid. ***p<.001, **p<.01, *p<.05 (Benjamini-Hochberg correction).

Figure 3: Cortisol awakening response (CAR) at time point 3h post-awakening/ 7.5 h post-GHB challenge placebo vs. GHB. **p<.01, *p<.05 (Benjamini-Hochberg correction).

Figure 4: Brain derived neurotrophic factor BDNF serum levels at time point 3h post-awakening/ 7.5 h post-GHB challenge placebo vs. GHB.

Table 1: Means, standard deviations (SD) of assessed metabolites, for the placebo and GHB condition (N=20). 3HK: 3-hydroxykynurenine, KYNA: kynurenic acid, QUIN: quinolinic acid. ***p<.001, **p<.01, *p<.05 (Benjamini-Hochberg correction).

Figure 1

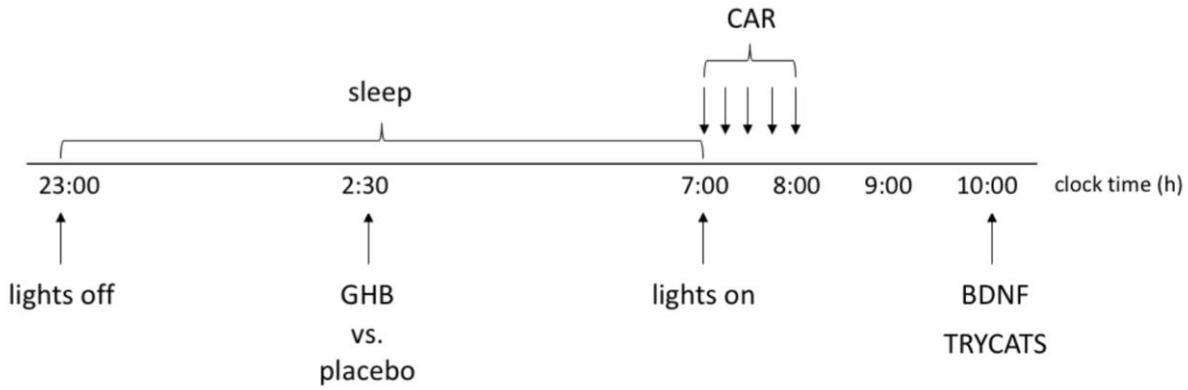

Figure 2

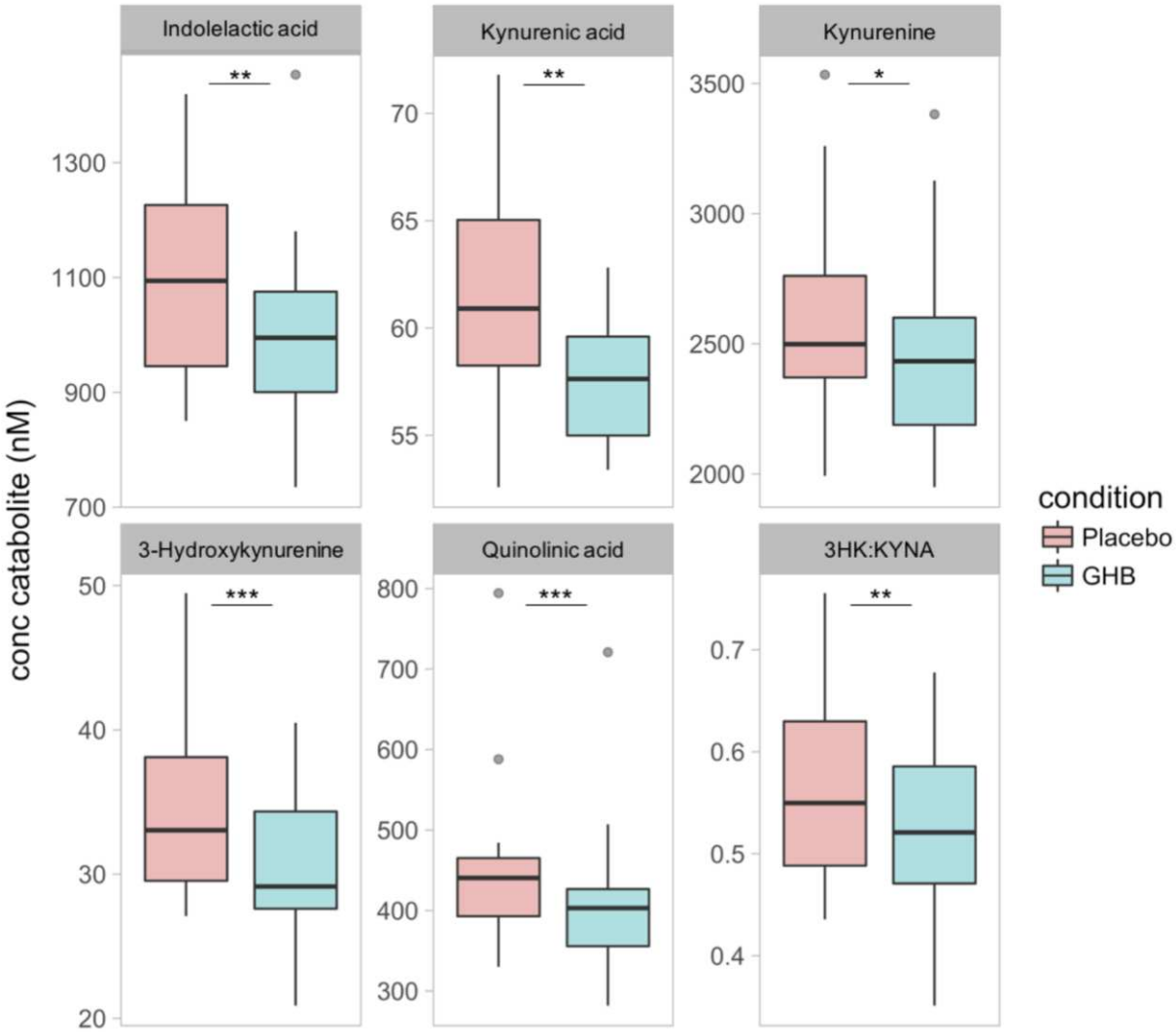

Figure 3

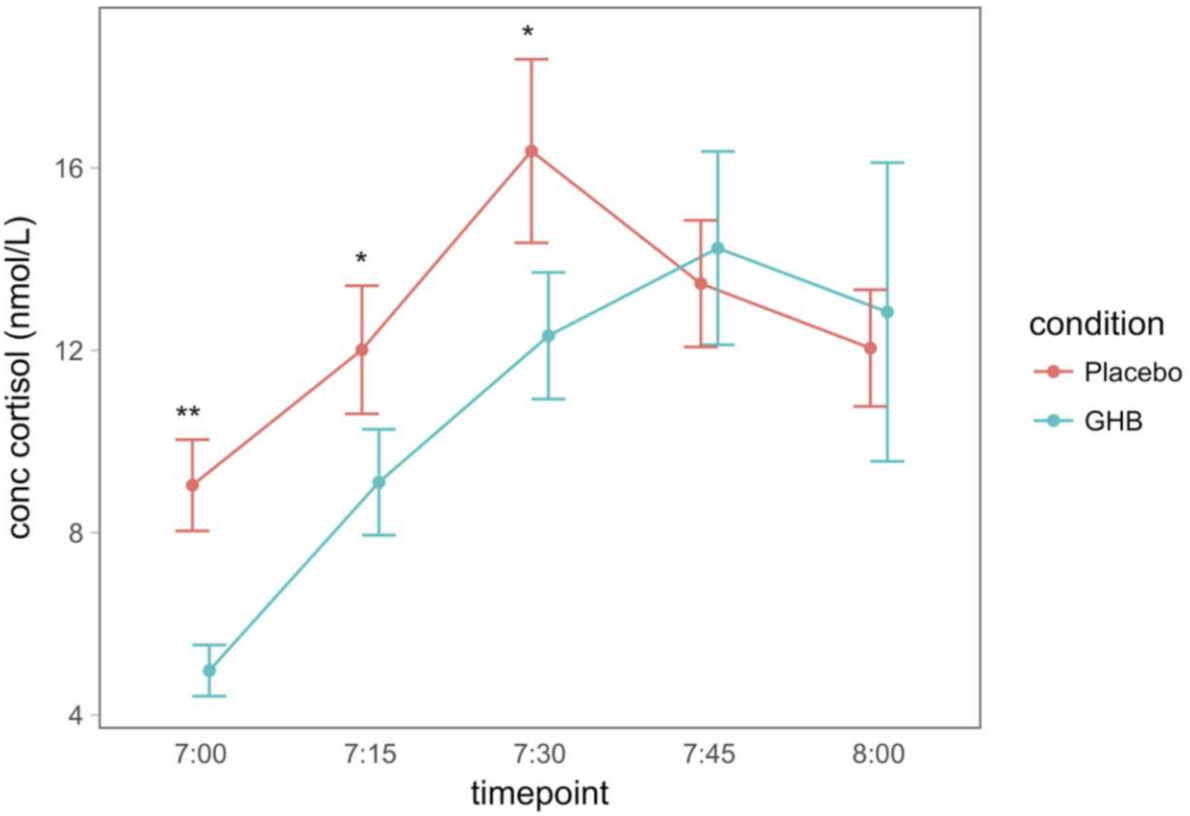

Figure 4